NTP Research Concept: Indium and Indium-Tin Oxide Toxicity

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Nomination Background and Rationale:
Indium-tin oxide (ITO, CASRN 50926-11-9) was nominated by the NIEHS due to a dramatic increase in world usage over the past fifteen years and because indium phosphide (InP, CASRN 22398-80-7), the indium compound most commonly used in commerce until the last decade, is carcinogenic in rats and mice when inhaled (NTP, 2001). One hypothesis generated by these studies is that elemental indium, potentially released after solubilization of InP in the lungs by macrophages or by an unknown mechanism, is the carcinogenic moiety. NTP studies on ITO will be designed to evaluate, in a comparative tiered testing strategy, its solubility *in vitro* (cell free) and *in vivo*, and its long-term toxicity in rodents exposed by inhalation. For comparison, indium chloride will be evaluated along with ITO. These studies will provide data for ITO risk assessment and answer fundamental questions about the toxicity of elemental indium.

Demand for indium has increased dramatically over the past 15 years, due largely to the growth of the electronics market. In 1996, the United States, which does not produce indium, imported 15 metric tons; by 2001-2005, imports had increased to 79 to 150 metric tons per year. The projected worldwide demand of indium for 2009 is 1555 metrics tons. Secondary indium production, which accounts for 63% of the 2009 indium supply, is derived from the reclamation of indium from ITO scrap. Eighty-two percent of world indium is used as ITO in products such as flat panel displays (FPD). Indium is also used in the production of indium phosphide (InP), which is used in the semiconductor industry.

ITO is a mixture of indium oxide (IO) and tin oxide (TO) in varying ratios, the most common being 90% IO and 10% TO. ITO is utilized by the electronics industry because it serves as a transparent electrical conductor and infrared reflector. The majority of ITO production is used in the electronics industry as a thin film coating on FPD liquid crystal displays and plasma displays. Other uses include field emission displays, heat reflective coatings, solar panels, cathode-ray tubes, energy efficient windows, gas sensors, photovoltaics, windshields, and alloys. Usage of ITO in the United States is expected to increase because of an increasing focus on green energy technology.

There are several technologies used to create ITO as well as to apply it to the substrate material of interest. ITO targets (i.e., solid blocks of ITO) are commonly created by sintering IO and TO at high temperatures. The ITO targets are then used to deposit a thin film of ITO on the desired substrate material via spray pyrolysis, sputtering, or other deposition technologies. The myriad of technologies used to create ITO targets and subsequent ITO thin film application on substrate materials have resulted in potential exposure to ITO particles ranging in size from nano to microparticles, as well as to varying ratios of IO and TO.

ITO target creation, ITO film deposition, and ITO recycling are the three critical areas for potential inhalation exposure during ITO production and use. Inhalation exposure can occur at several stages. The creation of ITO targets presents a risk to workers during polishing and cutting. ITO targets are used to create thin films of ITO on selected substrates via various deposition technologies. Only ~30% of the ITO target becomes attached to the desired target; the excess is reclaimed via recycling. Outdated electronics are also utilized in secondary indium production and represent another source of exposure to workers and potentially the general public. NIOSH has received funding in 2009 to perform an indium and indium compound exposure assessment in the United States. Initial data from the USA is expected in 2010. However, the majority of world ITO is utilized in East Asia.

Greater than 45% of ITO targets are manufactured in Japan and there have been several published clinical reports of workers adversely affected by exposure to ITO. The first clinical study, published in 2003, was of a wet surface grinder operator who developed interstitial pneumonia. ITO particles were detected throughout his lungs and he had an extremely high level of serum indium. Ultimately, he developed bilateral pneumothorax, which was the cause of death. Other case studies included a worker with pulmonary fibrosis and another with pneumothorax. Larger studies of workers occupationally exposed to ITO demonstrated interstitial pulmonary disorders, increased serum indium, and increased levels of KL-6. KL-6 is a serum biomarker produced by alveolar type II cells and is a marker of interstitial lung injury. Serum indium levels were positively correlated to lung changes detected using high-resolution computed tomography. These studies prompted the Japanese to increase worker protection in indium processing plants.

ITO instilled into the lungs of hamsters via intratracheal administration caused inflammatory cell infiltration, exudation, thickening of the alveolar wall, accumulation of alveolar macrophages, diffuse alveolar cell hyperplasia, and thickening of the pleura. In this same study, ITO toxicity was less severe than an equivalent dose of InP. An industry study in rats demonstrated persistent inflammatory reactions in the lung after intratracheal administration of ITO particles but no fibrotic changes. Another study in hamsters demonstrated that ITO administered intratracheally caused slight vacuolization in the seminiferous epithelium of the testicles.

Indium compounds are known to cause teratogenic effects in laboratory animals. Soluble indium injected intravenously caused fetal gross malformations and fetal cartilage malformation that were more severe in rats than mice. However, InP demonstrated no statistically significant differences in a teratology study performed by the NTP. There were isolated cases of sex reversal/hermaphrodites in the InP treated groups. However, particle kinetics and time to steady state may have masked the teratogenic effect of InP because the particles may not have been solubilized to free indium in the two week exposure window utilized in teratology studies. Studies with a soluble indium compound such as indium chloride may be better able to evaluate the teratogenic potential of the element indium.

Key Issues:

Issue 1: Solubility of Indium Compounds

It is not known if inhaled indium compounds are solubilized in the lung. Indium particles may be phagocytized by alveolar macrophages and solubilized in the acidic environment of the lysosome. NTP will need to evaluate the solubility potential of various indium compounds *in vitro (*cell free) and *in vivo*. NTP will determine if solubility differences of indium compounds explain differences in observed in vivo toxicity (such as InP>ITO>IO).

Issue 2: Sintered vs. non-sintered ITO

Recent research suggests that the sintering process used to generate ITO, which appears to alter the crystal structure, may enhance toxicity. Sintered ITO should be used in the NTP studies since that is the predominant form of indium in the workplace. Chemistry studies mentioned in **Issue 1** will investigate the solubility differences of various indium compounds. It is hypothesized that sintering increases the solubility of indium compounds, thus increasing the amount of free indium released into the lung after inhalation exposure. Both ITO and InP are sintered indium compounds.

Issue 3: Particle size

The exposure profile to ITO includes nano- and micro-particles with varying ratios of IO and TO due to the myriad of technologies utilized in ITO target creation and thin film deposition. Particle size and composition effects could alter the toxicokinetic properties of ITO after inhalation exposure thus affecting absorption, distribution, metabolism, clearance, and possibly tissue burden.

The most appropriate ITO particle size and IO/TO composition will have to be determined by understanding the ITO exposure profile. This may be determined by the NIOSH exposure assessment studies beginning in 2009, but will be limited to the USA exposure. Most of the world's ITO is utilized in East Asia. It was decided that the NTP should initially pursue studies using ITO microparticles in the respirable size range.

Issue 4: Developmental and reproductive toxicology of Indium compounds

Animal studies in hamsters have demonstrated that InP, InAs, and ITO all cause changes in the seminiferous epithelium of the testes. Clinical pathology on indium workers in ITO plants has demonstrated elevated levels of serum indium. The potential male reproductive toxicity of ITO needs to be addressed in NTP studies. If warranted, teratology studies should be performed using a soluble indium compound such as indium chloride. Indium particles may require a time period for solubilization which may skew the results of a two week teratology study.

Issue 5: ITO carcinogenicity potential

Inhaled InP is carcinogenic in both rats and mice (NTP, 2001). It is not known if ITO is also carcinogenic. However, the current potential for occupational exposure to ITO is currently greater than exposure to the known carcinogen InP. Inhalation exposure could occur during indium refining, ITO target creation, ITO thin layer deposition, and ITO reclamation.

Issue 6: Carcinogenic potential of free indium

It is hypothesized that indium is the carcinogenic moiety in InP-treated animals. If ITO causes cancer, it does not prove that indium is the responsible toxicant because of the presence of tin in ITO. Tin may contribute additively or synergistically with indium to cause cancer. Thus, a comparative study with a pure indium compound may answer fundamental questions about indium toxicity in the lung. Pure indium metal or indium chloride are two potential chemicals to be used in comparative studies with ITO. Indium chloride is preferable because it is soluble and does require solubilization by physiological processes. Indium metal may prove to be insoluble in the lung and resistant to physiological degradation.

Proposed Approach:

Tier 1: Chemistry of indium compounds

Specific Aim 1: Determine the relative solubility of indium compounds.

The solubility of ITO and other indium compounds (InP, indium oxide, indium metal and tin oxide) will be assessed *in vitro* (cell free) and *in vivo*. It is thought that the acidic environment of the lysosome may be a potential area where indium compounds are solubilized. Sintering may also make indium compounds more soluble in comparison to non-sintered compounds and chemistry will address this issue.

Tier 2: 14-day, 90-day subchronic and DART inhalation exposures

Specific Aim 2: Assess ITO and indium chloride toxicity in subchronic studies. Use subchronic data to set concentrations for potential chronic studies.

Initial 14 day studies with microparticle ITO and aerosolized indium chloride (similar particle sizes) will provide concentration assessments to be utilized in the 90-day sub-chronic studies.

Comparative 90-day standard toxicology studies using a microparticle ITO and aerosolized indium chloride are recommended. For the ITO study, both tin and indium will be quantified by ICP-MS in tissues, serum, urine, and feces. Standard endpoints such as pathology, hematology, clinical pathology, micronucleus, sperm motility, and vaginal cytology will be measured. Special attention will be paid to lung, kidney, liver, and testicular pathology. The same endpoints will be measured for the comparative indium chloride study and only indium will need to be monitored by ICP-MS.

Specific Aim 3: Assess indium compound effects on reproduction and development.

Developmental and reproductive studies may also be performed depending on the results obtained in the sub-chronic studies. Indium chloride may be a better candidate for developmental toxicology studies because it does not need to be solubilized like ITO particles.

Tier 3: Chronic inhalation exposure

Specific Aim 4: Assess long-term toxicity of ITO and indium chloride.

Based on the 90-day sub-chronic studies, chronic studies will be performed if necessary for ITO and indium chloride. Standard endpoints such as pathology, hematology, clinical

pathology, micronucleus, sperm motility, and vaginal cytology will be measured. Tissue burden of indium and tin will also be determined by ICP-MS. Any unusual findings in the 90-day sub-chronic will also be assessed.

Significance and Expected Outcome:
Results from a two-year inhalation study will determine the carcinogenic potential of ITO and indium chloride in the rodent model system. This data will be invaluable to risk assessment of a chemical that is being utilized in excess of 1000 metric tons worldwide. Information will also be produced with respect to the toxic potential of ITO and indium on the testicular tissue. A two-year study on ITO and indium chloride will also provide insight into indium compounds in general. Coupled with the carcinogenicity data on InP, the ITO and indium chloride data may lead to regulation of all indium compounds in a similar manner.

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